



Europäisches Patentamt  
European Patent Office  
Office européen des brevets



Publication number: **0 682 877 A1**

(12)

**EUROPEAN PATENT APPLICATION**  
published in accordance with Art.  
158(3) EPC

(21) Application number: **95902958.8**

(51) Int. Cl.<sup>8</sup>: **A23L 1/20**

(22) Date of filing: **14.12.94**

(86) International application number:  
**PCT/JP94/02103**

(87) International publication number:  
**WO 95/16362 (22.06.95 95/26)**

(30) Priority: **14.12.93 JP 313254/93**

(43) Date of publication of application:  
**22.11.95 Bulletin 95/47**

(84) Designated Contracting States:  
**AT BE CH DE DK ES FR GB GR IE IT LI LU MC  
NL PT SE**

(71) Applicant: **NICHIMO COMPANY LIMITED**  
**6-2, Otemachi 2-chome,**  
**Chiyoda-ku**  
**Tokyo 100 (JP)**  
Applicant: **Hishiroku, Co. Ltd.**  
**79, Rokuro-cho,**  
**Yamatooji Higashihaaru 2-chome,**  
**Matsubara-dori**  
**Higashiyama-ku**  
**Kyoto-shi,**  
**Kyoto 605 (JP)**

(72) Inventor: **TAKEBE, Minoru Nichimo Co., Ltd.**

**6-2, Otemachi 2-chome**  
**Chiyoda-ku**  
**Tokyo 100 (JP)**  
Inventor: **ANDO, Yoshio**  
**Nichimo Co., Ltd.**  
**6-2, Otemachi 2-chome**  
**Chiyoda-ku**  
**Tokyo 100 (JP)**  
Inventor: **KIKUSHIMA, Sunao Hishiroku Co.**  
**Ltd. 79, Rokuro-cho**  
**Yamatooji Higashihaaru 2-chome**  
**Matsubara-dori,**  
**Higashiyama-ku**  
**Kyoto-shi**  
**Kyoto 605 (JP)**

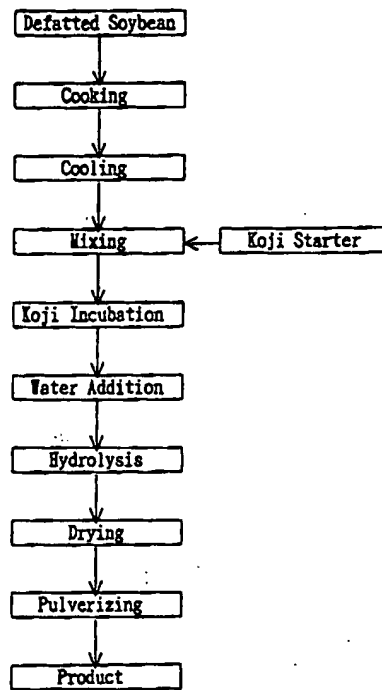
(74) Representative: **Pacitti, Pierpaolo A.M.E. et al**  
**Murgitroyd and Company**  
**373 Scotland Street**  
**Glasgow G5 8QA (GB)**

(54) **PROCESS FOR PRODUCING PRODUCT FROM PULSE AND FOOD CONTAINING PRODUCT FROM PULSE.**

(57) An efficient process for producing food, livestock feed and aquacultural feed which are excellent in the effects of preventing and suppressing cancer, treating osteoporosis and suppressing immune responses and can be taken in large quantities from leguminous grains or cakes. The obtained food such as biscuit has the abovementioned excellent pharmacological effects and is good for the health. It is possible to decompose a glycoside of an isoflavone compound contained in pulse to thereby generate an isoflavone compound containing the aglycon and to remove phytic acid present in pulse to thereby obtain a compound having excellent pharmacological effects.

**EP 0 682 877 A1**

Fig. 1



## Technical Field

The present invention relates to a process for preparing a product from a pulse crop as a starting material and a food containing the product prepared from a pulse crop as a starting material.

5 In the present invention, the term "pulse crop" means leguminous crops such as soybean, defatted products thereof and the like, and the term "product made from a pulse crop as a starting material" means foods, livestock feeds, aquacultural feeds and the like which are made from the above-mentioned pulse crop.

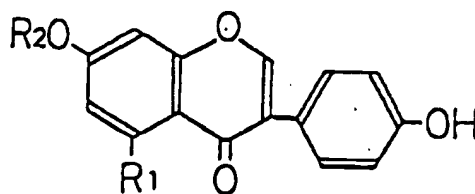
## 10 Background Art

In general, soybean which is one of the pulse crops contains isoflavone compounds including daidzin, daidzein, genistin and genistein.

The isoflavone compounds are represented by the following formula and Denotative Table.

15

20



25

30

Denotative Table		
	R1	R2
daidzin	H	glucose
daidzein	H	H
genistin	OH	glucose
genistein	OH	H

35

Of these isoflavone compounds, daidzein is an aglycone of daidzin having its glucose as a glycosidic saccharide hydrolytically separated therefrom, and genistein is an aglycone of genistin having its glucose as a glycosidic saccharide hydrolytically separated therefrom. With respect to the isoflavone compounds, contents thereof and percentages between daidzin and daidzein and between genistin and genistein in a

40 defatted soybean are as shown in the following Table 1.

Table 1

45

	daidzin	daidzein	genistin	genistein
defatted soybean	100 (96.9%)	3.2 (3.1%)	180 (97.7%)	4.2 (2.3%)
(unit: mg/100g)				

50 It is understood from Table 1 that, in soybean, daidzin and genistin are contained in larger amounts while daidzein and genistein which are aglycones thereof are contained in smaller amounts.

On the other hand, it has been reported that a glycosidic saccharide is hydrolyzed from an isoflavone compound contained in soybean to form an aglycone in the course of soy sauce or miso (fermented soybean paste) preparation [see Sho-Ken (Soy-research) by Kiyoshi Kihara, vol.16, No.5, page 190 (1990)].

55 According to this report, however, although hydrolysis of a glycosidic saccharide proceeds to some extent by cooking of a defatted soybean or in a koji preparation step, most of the saccharide has already hydrolytically been separated in soy sauce sediment, or soybean miso. Accordingly, it is difficult to employ these for a process for preparing a product from a pulse crop as a starting material.

Further, many reports have been made on pharmacological activities of aglycones derived from hydrolysis of glycosidic saccharides from isoflavone compounds.

For example, genistein has been ascertained to be a tyrosine kinase inhibitor (TK inhibitor). Since tyrosine kinase is essentially responsible for cancer-induction by an oncogene, carcinostatic activities of genistein as a TK inhibitor are confirmed and the effect thereof has drawn attention [Akiyama et al.: Biochemistry, vol.59, No.9, page 1016 (1987)].

Further, estrogenic activities of an isoflavone compound have also attracted attention and have been confirmed to have osteoporosis therapeutic effect and immunosuppressive effect. In particular, genistein which is an aglycone of an isoflavone compound has notable estrogenic activities, and this activities enable osteopenia (bone resorption) to be suppressed.

Accordingly, many proposals concerning isoflavone compounds contained in soybean have been made in Japanese Unexamined Patent Publication No.126,186/1987, Japanese Unexamined Patent Publication No.258,669/1989, Japanese Unexamined Patent Publication No.170,756/1993, and so on.

#### 15 Disclosure of Invention

According to the method described in Japanese Unexamined Patent Publication No.126,186/1987, however, most of the resulting isoflavone compounds are daidzin and genistin which each have a glycosidic saccharide, and aglycones are contained in the resultant in small amounts. Thus, it is impossible to obtain foods and the like which have excellent pharmacological effect as mentioned above.

The method described in Japanese Unexamined Patent Publication No.258,669/1989 is one which comprises hydrolytically separating a glycosidic saccharide from an isoflavone compound by action of  $\beta$ -glucosidase which is one of enzymes contained in soybean per se. However, aglycones are formed in a small proportion.

The method described in Japanese Unexamined Patent Publication No.170,756/1993 is one which comprises extractively separating isoflavone compounds from aglycones of isoflavones formed in a soy sauce sediment or soy sauce. Although aglycones of isoflavones are formed in the course of soy sauce preparation and yet formed in a very high proportion as described above, there is the following disadvantage. That is, aglycones of isoflavones are present in soy sauce sediment because of their insolubility and soy sauce sediment per se does not serve as a food, and hence the method cannot be employed as a method for preparing a food. Further, aglycones of isoflavones are also formed in a soybean miso at initial stage of preparation. However, a soybean miso has a problem that it should be avoided to ingest a soybean miso in a large amount because it is a highly salinized food.

Although ingestion of foods in a satisfactory amount which contain a sufficient amount of such isoflavone aglycones having excellent pharmacological activities as mentioned above enables dietarily desired life to be realized which exhibits excellent effect in terms of health maintenance of a human being, no food has heretofore satisfied this expectation.

Thus, the advent of foods which have excellent carcinopreventive and carcinostatic activities, osteoporosis therapeutic activities, and immunosuppressive activities, and which can be ingested in a satisfactory amount.

With respect to osteoporosis, it is desired to remove phytic acid, which inhibits calcium from being absorbed in a body, from a pulse crop.

In soybean which is one of beans, phytic acid is contained in an amount of about 1 to 2 % by weight. Phytic acid is residually present also in a product made from soybean and inhibits activities of a vitamin B complex contained in the product to prevent absorption of minerals and the like contained in the product. Further explanatively, phytic acid is a compound having such a structure that myo-inositol has its all hydroxyl groups each bonded with a phosphoric acid group, and chelates with a nutritionally important trace metal element to form hardly soluble compound. Accordingly, when a food with high phytic acid content is ingested by a human being or animal, normal intestinal absorption of such metals, for example, calcium, magnesium, iron, zinc and the like is prevented to cause various deficiencies. It has further been found that phytic acid present in a product including a soy protein isolate prevents a monogastric animal from utilizing zinc in a food. Further, phytic acid is known to have inhibitory activities on various digestive enzymes in a gastrointestinal digestive tract on which ions of minerals such as calcium act as activators and which include  $\alpha$ -amylase, pepsin and trypsin. It is, therefore, desired to remove phytic acid from the product.

Heretofore, however, it has been impossible to remove phytic acid successfully.

The present invention has been made in view of these points. It is, therefore, an object of the present invention to provide a process for preparing a product from a pulse crop such as a food, a livestock feed,

an aquacultural feed or the like, which is made from a pulse crop, which has excellent carcinopreventive and carcinostatic activities, osteoporosis therapeutic effect and immunosuppressive effect, and which can be ingested in a sufficient amount.

It is another object of the present invention to provide a healthful food, such as a biscuit or the like which contains a product that is made from a pulse crop and that has properties excellent in carcinopreventive and carcinostatic activities, osteoporosis therapeutic effect and immunosuppressive effect.

To attain the above object, the process of the present invention for preparing a product from a pulse crop as a starting material, as claimed in claim 1, comprises:

inoculating a koji starter on a pulse crop to prepare koji; and  
 adding water to the resultant from the koji preparation treatment to advance hydrolysis of a protein contained in the resultant;

wherein in the course of the koji preparation and the proteolysis, separation of glycosidic saccharides from isoflavone compounds contained in the pulse crop is advanced to form isoflavone compounds containing aglycones in a large amount, thereby obtaining a product from the pulse crop as a starting material.

Further, the process of the present invention for preparing a product from a pulse crop as a starting material, as claimed in claim 2, comprises:

inoculating a koji starter on a pulse crop to prepare koji; and  
 adding water to the resultant from the koji preparation treatment to advance hydrolysis of a protein contained in the resultant;

wherein in the course of the koji preparation and the proteolysis, removal of phytic acid is advanced in parallel with the separation of glycosidic saccharides from isoflavone compounds contained in the pulse crop to form isoflavone compounds containing aglycones in a large amount, thereby obtaining a product from the pulse crop as a starting material.

Still further, the food of the present invention containing a product prepared from a pulse crop as a starting material, as claimed in claim 3, contains a product prepared from a pulse crop as a starting material by steps of:

inoculating a koji starter on a pulse crop to prepare koji; and  
 adding water to the resultant from the koji preparation treatment to advance hydrolysis of a protein contained in the resultant;

wherein in the course of the koji preparation and the proteolysis, separation of glycosidic saccharides from isoflavone compounds contained in the pulse crop is advanced to form isoflavone compounds containing aglycones in a large amount, thereby obtaining a product from the pulse crop as a starting material.

Further, the food of the present invention containing a product prepared from a pulse crop as a starting material, as claimed in claim 4, contains a product prepared from a pulse crop as a starting material by steps of:

inoculating a koji starter on a pulse crop to prepare koji; and  
 adding water to the resultant from the koji preparation treatment to advance hydrolysis of a protein contained in the resultant;

wherein in the course of the koji preparation and the proteolysis, removal of phytic acid is advanced in parallel with the separation of glycosidic saccharides from isoflavone compounds contained in the pulse crop to form isoflavone compounds containing aglycones in a large amount, thereby obtaining a product from the pulse crop as a starting material.

The present invention is constructed as described above and hence exhibits the following extremely excellent effects.

According to the process of the present invention for preparing a product from a pulse crop as a starting material as claimed in claim 1, it is realized that koji mold is propagated in koji preparation by inoculating koji starter on a pulse crop to hydrolytically separate glycosidic saccharides from isoflavone compounds contained in the pulse crop, and hydrolysis of a protein contained in the resultant from the koji preparation treatment is advanced by adding water thereto in parallel with further hydrolytic separation of glycosidic saccharides from isoflavone compounds contained in the pulse crop to form isoflavone compounds containing aglycones in a large amount.

According to the process of the present invention for preparing a product from a pulse crop as a starting material as claimed in claim 2, it is realized that koji mold is propagated in koji preparation by inoculating koji starter on a pulse crop to hydrolytically separate glycosidic saccharides from isoflavone compounds contained in the pulse crop and concurrently therewith to remove phytic acid in the pulse crop, and hydrolysis of a protein contained in the resultant from the koji preparation treatment is advanced by

adding water thereto in parallel with further hydrolytic separation of glycosidic saccharides from isoflavone compounds contained in the pulse crop to form isoflavone compounds containing aglycones in a large amount and in parallel with further removal of phytic acid.

The koji mold as mentioned above produces various enzymes, and of these enzymes,  $\beta$ -glucosidase, 5 phytase, phosphatase and protease are utilized to hydrolytically separate glycosidic saccharides from isoflavone compounds and to remove phytic acid contained in a pulse crop.

The food of the present invention containing a product made from a pulse crop as claimed in claim 3 or 4 is a food containing the above-mentioned product made from a pulse crop in accordance with the process as claimed in claim 1 or 2, respectively. The food contains the product having excellent carcinopreventive 10 and carcinostatic activities, osteoporosis therapeutic effect, immunosuppressive effect and the like, and accordingly, it is capable of maintaining parson's health constantly good when ingested as a health food.

The present invention is constructed and functions as described above, and hence the product prepared in accordance therewith is derived from a pulse crop and is of excellence in carcinopreventive and carcinostatic activities, osteoporosis therapeutic effect, immunosuppressive effect and the like. Further, the 15 product is easy of digestion and yet easy of absorption because it is prepared through proteolysis. Accordingly, the product is nutritionally excellent in terms of protein utilization efficiency. In addition, the product can be used for a food, a livestock feed, an aquacultural feed and the like which may be ingested in a satisfactory amount, because no common salt has been added thereto.

Further, a food such as a biscuit which is so prepared as to contain the above-mentioned product is one 20 having excellent carcinopreventive and carcinostatic activities, osteoporosis therapeutic effect, immunosuppressive effect and the like, and accordingly, it is capable of maintaining parson's health constantly good when ingested as a health food. In particular, genistein in isoflavone compounds containing aglycones in a large amount obtained by hydrolytically separating glycosidic saccharides from isoflavone compounds is highly effective because of its high carcinopreventive and carcinostatic activities on mastocarcinoma, 25 prostatic cancer and the like.

#### BRIEF DESCRIPTION OF THE DRAWINGS

Fig.1 is a flow chart showing one mode of the process for preparing a product from a pulse crop 30 according to the present invention, which comprises forming aglycones having high pharmacological activities from isoflavone compounds contained in a defatted soybean, and one mode of the process which further comprises concurrently removing phytic acid contained in the defatted soybean.

Fig.2 is a diagram showing temperature characteristics of a mixture with progress of koji preparation time. 35

#### BEST MODE FOR CARRYING OUT THE INVENTION

Now, embodiments of the present invention will be described with reference to Fig.1.

Fig.1 is a flow chart showing one mode of the process for preparing a product from a pulse crop 40 according to the present invention, which comprises hydrolytically separating glycosidic saccharides from isoflavone compounds contained in a defatted product of soybean which is one of pulse crops to form isoflavone compounds containing aglycones in a large amount in the resulting product, and one mode of the process which further comprises concurrently removing phytic acid contained in the defatted soybean. In other words, by the same preparation procedure, the process according to claim 1 and the process 45 according to claim 2 of the present invention can be carried out.

In the first place, the invention according to claim 1, i.e., the process for preparing a product will be described, which comprises forming isoflavone compounds containing aglycones in a large amount.

Explanation will be given along the procedure in Fig.1. First, a defatted soybean is cooked. By effecting the cooking, propagation of koji is facilitated. The cooking of the defatted soybean may be conducted 50 batchwise or continuously according to the purpose of preparation or the like.

After completion of the cooking, the defatted soybean is once cooled to adjust water content in the defatted soybean to a level allowing koji to propagate (for example, 40 % by weight).

Incidentally, when a defatted soybean or the like is used as a starting material, the step of cooking may be omitted.

55 The defatted soybean thus adjusted in the water content is subjected to the process of the present invention as follows.

That is, the defatted soybean already cooked is inoculated with a koji starter of a koji mold at a predetermined weight ratio, and mixing is conducted to uniformness.

Then, the mixture is charged into a device for preparing koji and kept in a heated condition at an initial temperature of about 28 to 30 °C for a predetermined period of time to ferment the defatted soybean having a water content as low as 40 % by weight with koji, thereby hydrolytically separating glycosidic saccharides from isoflavone compounds contained in the defatted soybean to form aglycones. The koji preparation is continued until an enzyme necessary for hydrolytically separating the glycosidic saccharides from the isoflavone compounds.

In this stage, the koji is propagated on the defatted soybean to produce  $\beta$ -glucosidase which is an enzyme hydrolytically separating a glycosidic saccharide from an isoflavone compound, and by this enzyme, glycosidic saccharides are hydrolytically separated from the isoflavone compounds contained in the defatted soybean to form aglycones of the isoflavones.

As the koji starter for the koji preparation, there may be used those which are used preparation of Japanese traditional fermented foods and tempeh and which are safely used for foods, for example, those classified as *Aspergillus* genus such as *Aspergillus usamii*, *Aspergillus kawachi*, *Aspergillus awamori*, *Aspergillus saitoi*, *Aspergillus oryzae* and *Aspergillus niger*; and those classified as *Rhizopus* genus.

The fermentation time depends upon the type of koji mold used. However, it is at Least 24 hours and is appropriately selected to be sufficient one for hydrolytically separating glycosidic saccharides from the isoflavone compounds contained in the defatted soybean to satisfactory extent.

The temperature of the mixture in the device for preparing koji changes with time, for example, as shown in Fig.2, as koji preparation proceeds. That is, the temperature gradually rises until the state of "prime (Sakari)" is reached 22 hours after the initiation of the koji preparation, and the temperature slightly falls past the "prime". Then, the temperature rises again until the stage of "intermediary (Naka)" is reached 27 hours after the initiation of the koji preparation. Upon stirring the mixture at the "intermediary", the temperature slightly falls. Then, the temperature rises again until the stage of "maturity (Shimai)" is reached 32 hours after the initiation of the koji preparation. Upon stirring the mixture at the "maturity", the temperature slightly falls. Then, the temperature rises again up to 40 hours after the initiation of the koji preparation. Thereafter, the temperature gradually falls until the koji preparation reaches completion 48 hours after the initiation of the koji preparation.

Then, water is added to the product resulting from the koji preparation, and the mixture is kept in a heated condition at 30 to 65 °C for a predetermined period of time to hydrolyze protein while sufficiently separating glycosidic saccharides from the isoflavone compounds contained in the defatted soybean by the action of  $\beta$ -glucosidase contained in the product to form aglycones of isoflavones.

With respect to the hydrolysis of the protein, hydrolysis time and hydrolysis temperature are appropriately selected depending upon the type of koji used so that glycosidic saccharides are separated from the isoflavone compounds contained in the defatted soybean to satisfactory extent.

In this manner, organic acids are formed in the initial stage of the fermentation to inhibit contaminants in the defatted soybean from propagating, thereby eliminating undesired possibility of secondary contamination. Consequently, a product made from a defatted soybean as a starting material can be mass-produced. Further, even if the water content is not low, it is possible to carry out such treatment for separating glycosidic saccharides from the isoflavone compounds sufficiently.

Table 2 shows contents of isoflavone compounds in 100g of a defatted soybean which is prepared by subjecting an untreated defatted soybean to koji preparation initiated at an initial temperature of 30 °C and completed over a period of 48 hours, adding water to the resulting product in the same weight as that of the resulting product, and subjecting the mixture to hydrolysis of proteins at 30 °C for 24 hours.

Table 2

daidzin	daidzein	genistin	genistein
25	74	53	59
(unit: mg/100g)			

According to Table 2, daidzein and genistein which are aglycones of isoflavone compounds are contained in greatly increased amounts of 74mg and 59mg which are about 23 times and 14 times as large as the amounts thereof in the conventional example shown in Table 1, respectively. From this, it is understood that daidzein and genistein can be formed in further increased amounts by effecting the hydrolysis of proteins for 24 hours or more after the completion of the koji preparation.

In another Example, the treatment according to the process of the present invention was applied to an untreated defatted soybean and a soy protein isolate, and Table 3 comparatively shows, for the same purpose as that of Table 2, measurements thereon prior and posterior to the treatment.

Explanation is first made with respect to one of them, the defatted soybean. Proportions of starting materials and koji starter was such that 100g of a defatted soybean, 0.1g of a roughly polished rice, and  $8 \times 10^7$  koji spores/g were used. With such proportions, the untreated defatted soybean was subjected to koji preparation initiated at an initial temperature of 30 °C and completed over a period of 48 hours, and water was added to the resulting product, in the same weight as that of the resulting product, and the mixture was subjected to hydrolysis of proteins at 50 °C for 48 hours. The results are as shown in Table 3.

As the other of them, i.e., the commercially available soy protein isolate, Fujinic 200 (trade name) manufactured by Fuji-Purina k.K. was used. Proportions of starting materials and koji starter was such that 100g of the commercially available soy protein, 0.1g of a roughly polished rice, and  $8 \times 10^7$  koji spores/g were used. With such proportions, the untreated commercially available soybean protein was subjected to koji preparation initiated at an initial temperature of 30 °C and completed over a period of 48 hours, and water was added to the resulting product in the same weight as that of the resulting product, and the mixture was subjected to hydrolysis of proteins at 50 °C for 48 hours. The results are as shown in Table 3.

Table 3

	defatted soybean		commercially available soybean protein	
	pre	post	pre	post
daizin	100	not detected	90	1.0
daizein	3.2	70	5.3	100
genistin	120	1.3	120	3.3
genistein	4.2	64	4.4	94
(unit: mg/100g)				

According to Table 3, in the defatted soybean, daidzein and genistein which are aglycones of isoflavone compounds are contained in greatly increased post-treatment amounts of 70mg and 64mg which are about 22 times and 15 times as large as the pre-treatment values, respectively. In addition, daidzin which is an isoflavone compound having a glycosidic saccharide is decomposed to an undetectable extent, and the amount of genistin is extremely reduced to a level as low as 1.3mg.

Likewise, in the commercially available soybean protein, daidzein and genistein which are aglycones of isoflavone compounds are contained in greatly increased post-treatment amounts of 100mg and 94mg which are about 19 times and 21 times as large as the pre-treatment values, respectively. In addition, the amounts of daidzin and genistin which are isoflavone compounds each having a glycosidic saccharide are extremely reduced to a level as low as 1.0mg and 3.3mg, respectively.

As described above, according to the present invention, of isoflavone compounds contained in soybean, aglycones having high pharmacological activities can be prepared at extremely high formation ratios.

In the next place, the invention according to claim 2, i.e., the process for preparing a product will be described, which comprises forming isoflavone compounds containing aglycones in a large amount, and concurrently removing phytic acid contained in a defatted soybean.

The preparation procedure of the invention according to claim 2 is conducted in substantially the same manner as in the preparation procedure of the invention according to claim 1. However, in the koji preparation step, water addition step and hydrolysis step, phytic acid is removed from the defatted soybean in parallel with the formation of isoflavone compounds containing aglycones in a large amount.

Then, each of these steps will be described.

In the koji preparation step, a mixture of a defatted soybean and koji starter is charged into a device for preparing koji and kept in a heated condition at an initial temperature of about 28 to 30 °C for a predetermined period of time to ferment the defatted soybean having water content as low as 40% by weight by means of koji starter until phytic acid in the defatted soybean is sufficiently removed.

In this case, koji mold is propagated on the defatted soybean to produce phytase and phosphatase which are enzymes decomposing phytic acid, and by the enzymes, phytic acid in the defatted soybean is hydrolytically removed.

Specifically, from phytic acid which is a compound having such a structure that myo-inositol has all of its hydroxyl groups each bonded with a phosphoric acid group, the phytic acid-decomposing enzymes



liberate the phosphoric acid group(s) to form inositol pentaphosphate, inositol tetraphosphate, inositol triphosphate, inositol diphosphate, inositol monophosphate or inositol alone or a mixture thereof, thereby removing phytic acid.

As the koji starter for the koji preparation, there may be used koji molds which are used preparation of Japanese traditional fermented foods and tempeh and which are safely used for foods, for example, those having high phytase and phosphatase potency and classified as *Aspergillus* genus such as *Aspergillus usamii*, *Aspergillus kawachi*, *Aspergillus awamori*, *Aspergillus saitoi*, *Aspergillus oryzae* and *Aspergillus niger*; and those having high phytase and phosphatase potency and classified as *Rhizopus* genus.

The fermentation time depends upon the type of koji mold used. However, it is at least 24 hours and is appropriately selected to be sufficient one for removing phytic acid contained in the defatted soybean to satisfactory extent.

In the subsequent water addition step and hydrolysis step, water is added to the product resulting from the koji preparation, and the mixture is kept in a heated condition at 30 to 55 °C for a predetermined period of time to hydrolyze protein while sufficiently reducing the amount of phytic acid contained in the defatted soybean by the hydrolytic action of phytase, phosphatase and/or protease contained in the product.

With respect to the hydrolysis of protein, hydrolysis time and hydrolysis temperature are appropriately selected depending upon the type of koji used so that phytic acid contained in the defatted soybean is sufficiently removed.

The removal of phytic acid is effected by liberating at least one phosphoric acid group from phytic acid which is inositol hexaphosphate. In this connection, however, at least two phosphoric acid groups-liberated resultants, i.e., inositol tetraphosphate, inositol triphosphate, inositol diphosphate, inositol monophosphate and inositol are water-soluble and have activities to greatly facilitate absorption of a mineral such as calcium contained in a product made from a cereal.

Further descriptively, the above-mentioned inositol hexaphosphate and inositol pentaphosphate have strong ion capturing activities and prevent captured calcium ion from being liberated, thereby strongly inhibit absorption of calcium. On the other hand, inositol tetraphosphate to inositol monophosphate have such preferable affinities that they preferably capture calcium but readily liberate captured calcium on occasion, thereby exhibiting characteristic activities to facilitate absorption of calcium.

It is, therefore, preferred to effect removal of phytic acid by liberating at least two phosphoric acid groups from phytic acid which is inositol hexaphosphate to form inositol tetraphosphate, inositol triphosphate, inositol diphosphate, inositol monophosphate or inositol alone or a mixture thereof, thereby obtaining a product which enables minerals to be absorbed efficiently. In this case, it is preferred to control the number of the phosphoric acid groups liberated from phytic acid by adjusting the fermentation time, and hydrolysis time and hydrolysis temperature depending upon the type, state, properties and amount of the pulse crop, the type, state, properties and amount of the koji, and type and properties of the intended product.

Table 4 shows phytic acid content in 100g of a defatted soybean, with respect to an untreated defatted soybean; defatted soybeans A and B which are prepared using two different shochu kojis (*Aspergillus niger* and *Aspergillus awamori*) and each prepared by subjecting a defatted soybean to koji preparation initiated at an initial temperature 30 °C and completed over a period of 48 hours, adding water to the resulting product in the same weight as that of the resulting product, and subjecting the mixture to hydrolysis of protein at 30 °C for 24 hours; and a defatted soybean subjected to conventional washing treatment with an alcohol.

Table 4

defatted soybean	phytic acid content (mg/100g)
untreated defatted soybean	999 (mg/100g)
shochu koji-treated d. s. A	not detected
shochu koji-treated d. s. B	not detected
alcohol-washed d. s.	1,150 (mg/100g)
(detection limit: 5mg/100g)	

According to Table 4, in contrast to the phytic acid content of 999mg (about 1%) in the untreated defatted soybean, no substantial phytic acid contents in the defatted soybeans A and B are detected, which are each prepared according to the present invention by subjecting an defatted soybean to shochu koji

treatment, adding water to the resulting product in the same weight as that of the resulting product, and subjecting the mixture to hydrolysis of proteins at 30 °C for 24 hours. In other words, almost all phytic acid is decomposed in each of the defatted soybeans A and B.

On the other hand, the phytic acid content in the defatted soybean subjected to the conventional washing treatment with an alcohol is 1,150mg and no reduction of the phytic acid content is observed.

As described above, according to the present invention, of isoflavone compounds contained in soybean, aglycones having high pharmacological activities can be prepared at extremely high formation ratios, and at the same time, phytic acid content in soybean can be greatly or almost completely reduced.

Next, a food containing the product made from a pulse crop according to the present invention will be described.

The food containing the product made from a pulse crop according to the present invention includes a food consisting only of the product made from a pulse crop which is prepared in accordance with the process of the present invention and a food containing the product in part.

The product made from a pulse crop as a starting material which is prepared in accordance with the process of the present invention is a food having an extremely low salinity, because it is prepared without being salified with common salt. Accordingly, the product can be ingested in a sufficient amount when served as a food. And yet, the food contains aglycones of isoflavones in a large amount, which exhibit excellent carcinopreventive and carcinostatic activities, osteoporosis therapeutic effect and immunosuppressive effect, thereby enabling dietarily desired life to be realized which exhibits excellent effect in terms of health maintenance of a human being.

For example, when the food containing the product made from a pulse crop according to the present invention is formed into a form convenient for eating such as a biscuit, cookie or the like, it is possible to ingest aglycones of isoflavones which have excellent carcinopreventive and carcinostatic activities, osteoporosis therapeutic effect and immunosuppressive effect while such an article is eaten as a food. In particular, by simply eating such a biscuit or the like in an amount covering the intake of aglycones of isoflavones per day which is required to attain carcinopreventive and carcinostatic effect, osteoporosis therapeutic effect and immunosuppressive effect, the biscuit or the like contributes to prevention of outbreak of the disorders.

Of these aglycones of isoflavones, genistein is effective for prevention and carcinostasis at an initial stage of mastocarcinoma, prostatic cancer and the like. Accordingly, ingestion of the food containing the product made from a pulse crop according to the present invention contributes to prevention of outbreak of these cancers, thereby enabling dietarily desired life in terms of health maintenance to be realized.

Further, with respect to osteoporosis, while aglycones of isoflavones exhibit osteopenia preventive effect, the removal of phytic acid enables a vitamin B complex having growth promoting activities and anti-lipidhepatic activities and the like to be maintained highly active and hence exhibits facilitative effect on absorption of calcium contained in the pulse crop. Moreover, these effects synergistically provide a food having extremely excellent osteoporosis therapeutic effect. In particular, such a food exhibits significant effect when used in dietotherapy for a person hormone-relatedly susceptible to osteoporosis.

When the defatted soybean prepared in accordance with the above-described procedure is utilized as a feed or the like, as shown in Fig.1, the defatted soybean prepared as in the above-described embodiments is dried and then pulverized to obtain a product as a pulverized defatted soybean having high pharmacological activities, such as a material for a livestock feed, an aquacultural feed or the like.

According to the present invention, formation of aglycones of isoflavone compounds contained in a pulse crop, which have high pharmacological activities, at an extremely high formation ratio; removal of phytic acid in the pulse crop; and hydrolysis of proteins are effected, by propagation of living koji. Therefore, the formation of aglycones and the removal of phytic acid can be attained even if the pulse crop is in solid state or fluid state, thereby enabling simplified preparation procedure and reduced preparation cost to be realized.

Further, the preparation process of the present invention can be carried out using a conventional device for preparing koji without any alteration, and hence a basic device for production is not required to be specially manufactured, thereby providing wide utility.

It is to be noted that the present invention is by no means restricted to the above-described embodiments and that various alterations and modifications can be made according to need.

## 55 Claims

1. A process for preparing a product from a pulse crop as a starting material which comprises:  
inoculating a koji starter on a pulse crop to prepare koji; and

adding water to the resultant from the koji preparation treatment to advance hydrolysis of a protein contained in the resultant;

wherein in the course of the koji preparation and the proteolysis, separation of glycosidic saccharides from isoflavone compounds contained in the pulse crop is advanced to form isoflavone compounds containing aglycones in a large amount, thereby obtaining a product from the pulse crop as a starting material.

2. A process for preparing a product from a pulse crop as a starting material which comprises:

inoculating a koji starter on a pulse crop to prepare koji; and

adding water to the resultant from the koji preparation treatment to advance hydrolysis of a protein contained in the resultant;

wherein in the course of the koji preparation and the proteolysis, removal of phytic acid is advanced in parallel with the separation of glycosidic saccharides from isoflavone compounds contained in the pulse crop to form isoflavone compounds containing aglycones in a large amount, thereby obtaining a product from the pulse crop as a starting material.

3. A food containing a product prepared from a pulse crop as a starting material, the product being prepared by steps of:

inoculating a koji starter on a pulse crop to prepare koji; and

adding water to the resultant from the koji preparation treatment to advance hydrolysis of a protein contained in the resultant;

wherein in the course of the koji preparation and the proteolysis, separation of glycosidic saccharides from isoflavone compounds contained in the pulse crop is advanced to form isoflavone compounds containing aglycones in a large amount, thereby obtaining a product from the pulse crop as a starting material.

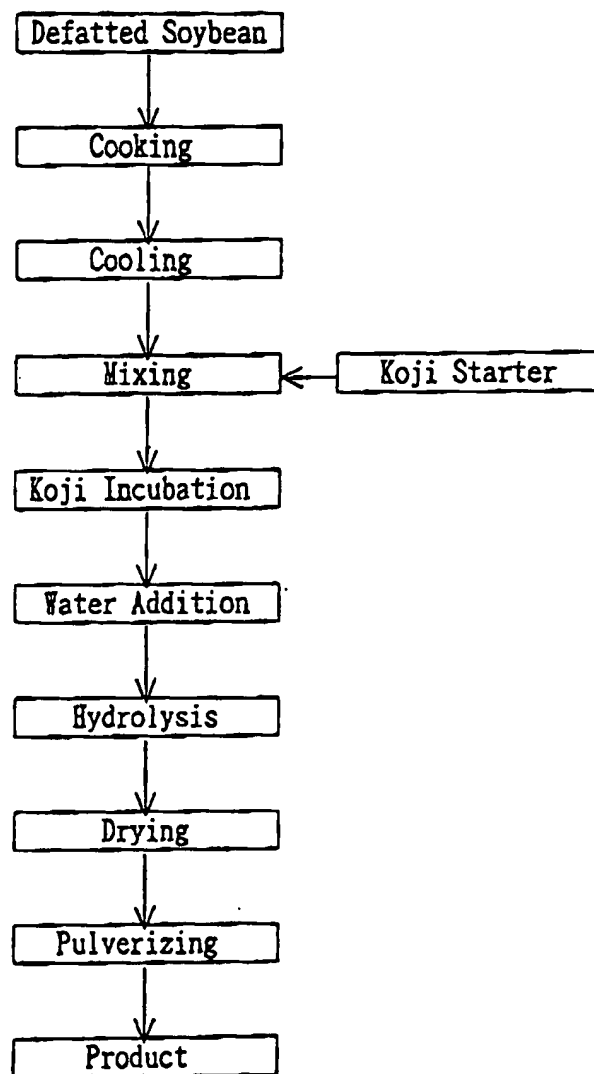
4. A food containing a product prepared from a pulse crop as a starting material, the product being prepared by steps of:

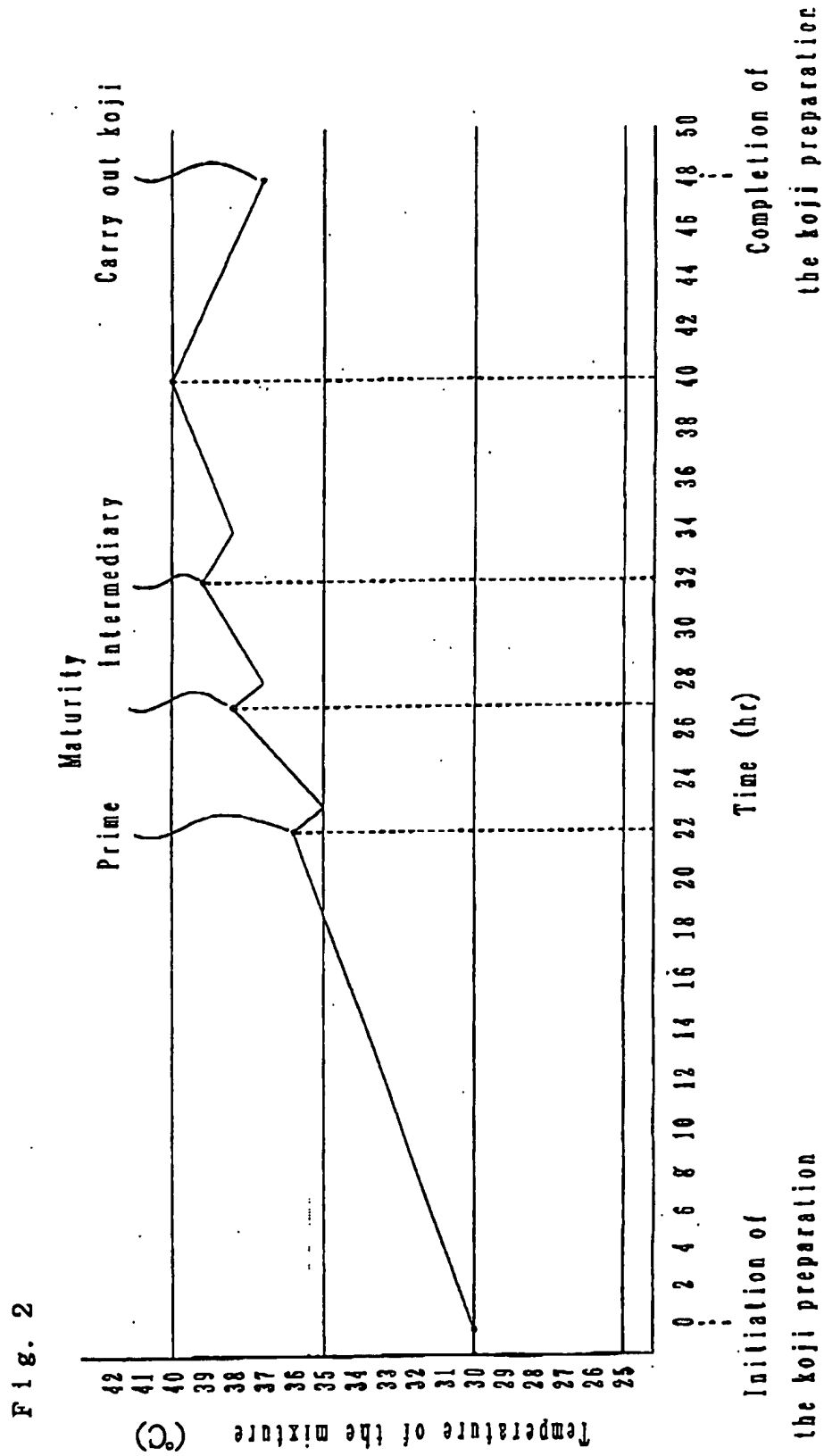
inoculating a koji starter on a pulse crop to prepare koji; and

adding water to the resultant from the koji preparation treatment to advance hydrolysis of a protein contained in the resultant;

wherein in the course of the koji preparation and the proteolysis, removal of phytic acid is advanced in parallel with the separation of glycosidic saccharides from isoflavone compounds contained in the pulse crop to form isoflavone compounds containing aglycones in a large amount, thereby obtaining a product from the pulse crop as a starting material.

F i g . 1





## INTERNATIONAL SEARCH REPORT

International application No.

PCT/JP94/02103

<b>A. CLASSIFICATION OF SUBJECT MATTER</b> Int. C1 <sup>6</sup> A23L1/20 According to International Patent Classification (IPC) or to both national classification and IPC		
<b>B. FIELDS SEARCHED</b> Minimum documentation searched (classification system followed by classification symbols) Int. C1 <sup>6</sup> A23L1/20 Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)		
<b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b>		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
PX	JP, A, 5-328929 (Fujikko K.K.), December 14, 1993 (14. 12. 93), Claim (Family: none)	1-4
X	JP, A, 51-125771 (Fuji System K.K.), November 2, 1976 (02. 11. 76), Claim (Family: none)	1-4
Y	JP, A, 59-166049 (Nippon Shinyaku Co., Ltd.), September 19, 1984 (19. 09. 84), Claim & KR, B, 9000960	2, 4
<input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/> See patent family annex.		
* Special categories of cited documents: "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier document but published on or after the international filing date "L" document which may throw doubt on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family		
Date of the actual completion of the international search February 14, 1995 (14. 02. 95)		Date of mailing of the international search report March 7, 1995 (07. 03. 95)
Name and mailing address of the ISA/ Japanese Patent Office Facsimile No.		Authorized officer Telephone No.